Reversion of Kirsten Sarcoma Virus Transformed Human Cells: Elimination of the Sarcoma Virus Nucleotide Sequences

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SUMMARY

The virus-specific nucleotide sequences in the RNA and DNA of a Kirsten mouse sarcoma virus (Ki-MSV)-transformed non-producer human osteosarcoma cell clone and two subclones of these cells that reverted to a normal phenotype have been analysed by hybridization of sarcoma virus-specific complementary DNA (cDNA) to cellular RNA or DNA. Whereas the transformed clone had acquired de novo Ki-MSV sequences in the RNA and DNA of the cells, both the revertant cell lines seemed to have lost most or all of this information from the cellular nucleic acids. The DNA from the revertant cells lacked the sequences represented either in the Ki-MSV-specific cDNA or in the total cDNA of the leukaemia-sarcoma virus complex. Thus, the reversion of the virus-transformed human cells to normal morphology is associated with the loss of most or all of the proviral sequences from the cellular DNA.

It is generally accepted that in tumour virus-infected cells the expression of virus genes influences cell growth characteristics. The expression of virus transforming genes is essential for maintenance of cellular transformation but it is the expression of a combination of virus and cellular genes that determines the phenotype of a transformed cell. Consequently, variant cells can be isolated which have acquired a normal phenotype but still contain virus genes (Pollack et al. 1968; Rabinowitz & Sachs, 1968; Stephenson et al. 1972). The level of virus gene transcripts can be reduced or can remain the same in revertant cells (Deng et al. 1974, 1977; Krzyzek et al. 1977). Often the variants show an increased number of chromosomes, suggesting the importance of chromosomal balance as well as the presence of virus genes for transformation (Gazdar et al. 1974; Nomura et al. 1972; Pollack et al. 1970; Rabinowitz & Sachs, 1970). Another mechanism for reversion is the loss of integrated virus genes from the cellular DNA. An example of this type of reversion is that when cells abortively transformed by SV40 virus return to normal they no longer contain the virus DNA (Dulbecco, 1976). Elimination of the sarcoma virus nucleotide sequences from the DNA of revertant cell lines derived from murine sarcoma virus transformed clonal cat and mouse cells has also been reported (Frankel et al. 1976; Bensinger et al. 1977). Our present study of the Kirsten mouse sarcoma virus (Ki-MSV)-transformed non-producer cell clone of human osteosarcoma (KHOS; Rhim et al. 1975) and its revertant subclones (KHOS-312H and KHOS-240S; Cho et al. 1976) provides additional examples of the reversion mechanism by loss of virus information.

The Kirsten strain of mouse sarcoma virus (Ki-MSV) and mouse erythroblastosis virus (MEV) were isolated from a normal rat kidney (NRK) cell line (No. 58967), transformed and productively infected with Ki-MSV, and an NRK cell line productively infected with MEV, respectively, as described by Roy-Burman & Klement (1975). This Ki-MSV stock showed a purity of at least 90% for the transforming particles. The term MEV has been used to indicate the original mouse leukosis virus that gave rise to Ki-MSV after in vivo propagation in rats. The non-focus-forming virus (helper) of Ki-MSV stocks, designated
Ki-MuLV, was also propagated in NRK cells. MEV and Ki-MuLV are very closely related if not identical (Roy-Burman & Klement, 1975). The rat leukaemia virus (RaLV) was isolated from Sprague-Dawley rat embryo fibroblast cells spontaneously releasing the virus (Rasheed et al. 1976). The rat cell lines and the human osteosarcoma cell clones non-productively transformed by Ki-MSV (KHOS) and its two revertant subclones (KHOS-312H and KHOS-240S; Cho et al. 1976) were grown in Eagle's minimum essential medium and 10% foetal bovine serum. Fischer rat tumour transplants of Ki-MSV transformed rat cells (No. T-84710) were kindly provided by Dr R. Huebner of NCI.

A 3H-labelled complementary DNA (cDNA) probe was prepared from disrupted virions of Ki-MSV in the presence of actinomycin D (Niman et al. 1977b). This cDNA preparation, representing sequences complementary to the Kirsten leukaemia-sarcoma virus RNA sequences, will be referred to as Ki-MSV total cDNA. Preparation of a cDNA free of helper virus sequences and, thus, specific for the Ki-MSV genome was needed to enhance the detectability of Ki-MSV nucleic acids in order to generate data concerning the presence or absence of sarcoma virus information in the revertant cells. The sarcoma virus-specific 3H-cDNA fraction (3H-cDNA<sub>spec</sub>) was isolated from the Ki-MSV total 3H-cDNA by hybridization to an excess of MEV poly(A)-containing RNA and selection of non-hybridizing portion by hydroxylapatite chromatography (Stehelin et al. 1976). The single-stranded cDNA fraction thus isolated, hybridized specifically to Ki-MSV poly(A)-containing RNA and not to poly(A)-containing RNAs of MEV, Ki-MuLV or RaLV. The normal rat DNA was found to contain sequences represented in the selected probe. These results were consistent with the presence of two major sets of sequences in Ki-MSV, one related to murine leukaemia virus, the other to rat cellular DNA (Scolnick et al. 1973; Roy-Burman & Klement, 1975; Shih et al. 1978).

Concentrations of RNA complementary to Ki-MSV-specific cDNA were measured in a number of normal and transformed cells. The C<sub>t</sub> (where C<sub>t</sub> is the concentration of RNA nucleotides in moles/l and t is the time in s) curves generated are shown in Fig. 1. The highest amount of MSV-specific RNA was detected in NRK cells productively transformed by

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**Fig. 1.** Kinetics of hybridization of Ki-MSV-specific cDNA to RNA from various cells and tissues. Purification of whole-cell RNA and hybridization assays using S<sub>i</sub> nuclelease were described (Niman et al. 1977a). C<sub>t</sub> values reported were corrected to 0.18 M-monovalent cation concentration (Britten & Smith, 1970). Sources of RNA were NRK cells productively transformed with Ki-MSV (▼), transplants of Ki-MSV transformed rat cells (No. T-84710) (●), KHOS cells (○), KHOS-312H cells (■), KHOS-240S cells (▲), and Sprague-Dawley rat kidney tissues (□). Curves generated with RNA from Sprague-Dawley rat spleen and embryo tissues were almost identical to that shown with kidney RNA (data not included).
Fig. 2. Reassociation kinetics between DNA from transformed and revertant cells and Ki-MSV total cDNA or selected Ki-MSV-specific cDNA. Purified cellular DNA, fragmented to 6S to 8S, was hybridized at 68 °C with 1H-labelled cDNA in 10 μl reaction mixtures containing 600 to 800 cti/min (8 to 10 pg) of cDNA and 50 μg of cell DNA in 0·75 M-NaC1, 0·02 M-tris-HCl, pH 7·2, 0·05 mm-EDTA and 0·05 % SDS in 50 μ1 sealed capillary tubes for various time periods ranging from 10 min to 40 h. Hybridization was assayed as described (Niman et al. 1977a) using 1 μg S1 nuclease and C0t values (where C0 is the concentration of DNA nucleotides in moles/l and t is the time in s) were corrected to 0·18 M-monovalent cation concentration (Britten & Smith, 1970). Hybridization between total 1H-cDNA and DNA from NRK cells productively transformed by Ki-MSV (○), KHOS cells (▲), KHOS-312H cells (△), KHOS-240S cells (×), and a cat lymphoma tissue (●) is shown in (b). Curves generated with DNA from human osteosarcoma (HOS) and rhabdomyosarcoma (RD) cells were identical to that shown with the revertant cell DNA (data not included). Hybridization between 1H-cDNAspec and DNA from KHOS cells (○), KHOS-312H cells (●), and KHOS-240S cells (△) is shown in (a).

Ki-MSV (Ct0.2 = 1·4 × 103), followed by a tumour transplant derived from Ki-MSV transformed rat liver epithelial cells (Ct0.2 = 4·8 × 103). A significant amount of this RNA was also detected in non-producer Ki-MSV transformed human osteosarcoma cells (KHOS) (Ct0.2 = 9·2 × 103), whereas the revertant clones (KHOS-312H and KHOS-240S) and normal rat tissues tested contained little or undetectable quantities of sarcoma virus-specific RNA. Hybridization kinetics using DNA from Ki-MSV-transformed NRK cells, KHOS and the revertants (KHOS-312H and KHOS-240S) are shown in Fig. 2. KHOS cell DNA showed a significant hybridization to Ki-MSV total cDNA, although the reassociation kinetics were an order lower than that observed with the DNA from the virus transformed NRK cells (Fig. 2b). There are several possible explanations to account partially for the substantially lower value for the final DNA–DNA hybridization with KHOS DNA, such as (i) the presence of a low proviral copy number, (ii) the presence of spontaneously reverted cells not readily visible but mixed with the transformed populations and (iii) contamination of cDNA preparations by non-viral sequences as the cDNA was not selected positively by hybridizing back against RNA from Ki-MSV grown in a heterologous cell. Although contributions by factors in (ii) and (iii) to the low levels of hybridization achieved in both RNA–DNA and DNA–DNA reactions could not be excluded, the
major difference might be related to the number of copies of the proviral DNA. Data from another laboratory indicated that while Ki-MSV-transformed NRK cells contained 20 to 30 copies of the virus genome per haploid cell genome, KHOS cells contained only a single copy per haploid genome (N. Tsuchida, personal communication). The results with the DNA from the KHOS-312H and KHOS-240S cells clearly indicated that both revertant cell clones lacked the sequences that could be detected in the parent KHOS cells by Ki-MSV total cDNA (Fig. 2b). Similarly, using the Ki-MSV-specific selected probe it was found that the DNA of both revertant lines lacked the sarcoma virus-specific sequences while these sequences were present in the transformed KHOS cell DNA (Fig. 2a).

The clone of non-producer human osteosarcoma cells transformed by Ki-MSV (KHOS cells) does not produce infectious helper virus or its antigens, but the cells contain the MSV genome which can be rescued by superinfection with MuLV (Rhim et al. 1975). The two revertants that have been analysed for the content of virus information in the present study were isolated from the KHOS cells after incubation at high temperature (40-5 °C) overnight and subcloning at 36 °C (Cho et al. 1976). These morphological variants, KHOS-312H and KHOS-240S, from which MSV could no longer be rescued, exhibit growth properties similar to those of the non-transformed, parent human osteosarcoma (HOS) cells. The revertants are non-tumorigenic in nude mice, whereas the KHOS cells are tumorigenic. The modal chromosome numbers of the revertant cells are similar to those of their progenitor KHOS cells (Cho et al. 1976).

Because the KHOS-312H and KHOS-240S revertants were obtained from progeny of single transformed KHOS cells, each cell of the KHOS clone that contains the sarcoma virus genome probably maintains the capacity to revert to normal phenotype. Diverse mechanisms may be responsible for generation of revertants, but the two subclones we tested indicate a common phenomenon. An examination of the presence of nucleotide sequences in RNA and DNA related to the transforming virus genetic sequences in transformed and revertant KHOS cells suggests that the transforming virus genome which was introduced into KHOS cells by infection has been lost from the cellular genome of both the revertant subclones. Whereas the KHOS non-producer clone has retained virus sequence in the cell DNA, the revertants no longer harbour these virus-specific sequences in their cellular DNA. The sarcoma virus-specific sequences are transcribed into RNA in KHOS cells and these transcripts are not detected in the revertant cells consistent with the lack of these nucleotide sequences in their DNA. Although the process by which both the subclones have returned to normality appears to be similar, the mechanism for loss of the transforming virus genome remains to be elucidated. Further studies on the nature of association of the provirus information with chromosomal DNA in the KHOS cell may provide clues to this type of reversion mechanism by elimination of the proviral DNA.

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